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Review on the Role of Dietary Zinc in Poultry Nutrition, Immunity, and Reproduction

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ABSTRACT

Zinc is an important nutrient in animal metabolism. In poultry, zinc serves not only as a nutrient but can also be used as a dietary supplement to manipulate the reproductive system of the bird. This article summarizes the general biochemistry, physiology, and nutritional aspects of zinc metabolism to provide a brief overview on what is known regarding zinc. The potential role of zinc in poultry immune response, *Salmonella* infection, and molting are emphasized.

Index Entries: Zinc; poultry; metabolism; nutrition; molting; immunology; uptake.

ZINC BIOCHEMISTRY

Zinc is ubiquitous in all living organisms and acts both structurally and catalytically in metalloenzymes *(1).* Zinc metalloenzymes are recognized in all six enzyme types, which include oxidoreductase (catalyzing oxidoreductions between two substrates), transferase (catalyzing transfer of a group other than hydrogen), hydrolase (catalyzing hydrolysis of esters,

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ether, peptide, glycosyl, acid anhydride, C–C, C–halide, or P–N bonds), lyase (catalyzing removal of groups from substrates by mechanisms other than hydrolysis, leaving double bonds), isomerase (catalyzing interconversion of optical, geometric, or positional isomers), and ligase (catalyzing the linking together of two components coupled to the breaking of a pyrophosphate bond in ATP or a similar compound) *(2).* These well-characterized zinc metalloenzymes are discussed in the following section.

Carbonic anhydrase plays a role in the transport of carbon dioxide from tissues to lungs and the zinc component is responsible for coordinating three imidazole groups *(2,3).* Superoxide dismutase plays an important role in protecting cells and tissue from damage by superoxide radical ($2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$) *(4,5).* Alcohol dehydrogenases catalyze the oxidation of ethanol, vitamin A, alcohol, and steroids using NAD as a cofactor and the reduction of aldehydes and ketones in the presence of NADH ($CH_3CH_2OH + NAD \rightarrow CH_3CHO + NADH + H^+$) *(6,7).* Each subunit of this enzyme contains two zinc atoms and binds one molecule of NAD(H). One zinc atom is essential for the catalytic activity and the other atom is involved in stabilizing the polymeric structure *(8).* The catalytic zinc atom is liganded in a tetrahedral geometry to two cysteinyl sulfurs, the imidazole group of histidine, and a water molecule *(9).* DNA and RNA polymerases are nucleotidyl transferases that catalyze the replication and transcription of the cellular genome. DNA polymerase and the synthesis of DNA are inhibited by the presence of EDTA *(10,11).* DNA and RNA polymerase are decreased in cell cultures when zinc chelators are added *(12).* In addition, zinc can influence gene expression by altering DNA and chromatin structure and is important for maintaining nucleic acid integrity *(13).* These observations indicate that inadequate zinc can result in damaged cellular functions caused by either decreased DNA or RNA synthesis, or gene expression. Carboxypeptidase A catalyzes the hydrolysis of aromatic amino acids, such as phenylalanine, tyrosine, or tryptophan, or the branched aliphatic amino acids *(14).* Carboxypeptidase B catalyzes hydrolysis of the basic amino acids, lysine, arginine, and ornithine from the carboxyl-terminal peptide bonds in polypeptides *(15).*

ZINC AND CELLULAR IMMUNITY

Inadequate intracellular concentrations of zinc cause abnormal development of T-lymphocytes in humans *(16)* and cause lower weights of the spleen and thymus mice (mice fed 5 ppm) when compared to control (mice fed with 100 ppm) *(17).* Involution of the thymus and small spleen weights are characteristics of zinc deficiency and appear to be primarily the result of an absence of white cells *(16,18,19).* In addition, zinc-deprived mice demonstrated significant growth retardation as reflected by lower body weight *(17).* Inadequate intracellular concentration of zinc also causes damage to the lymphocyte function that is responsible for the ability of T- and B-cell proliferation *(19).* Zinc deficiency can depress the DNA synthesis or

cell devision required for normal organ development because zinc is a structural component of many metalloenzymes, including those involved in gene replication, such as DNA and RNA polymerases *(20).* The activity of deoxythymidine kinase as a zinc-dependent enzyme is greatly reduced when the concentrations of zinc are deficient. This reduction is accompanied by decreased protein and collagen synthesis in rats *(20).* Impaired DNA synthesis results from zinc deficiency both in mammalian culture *(10)* and in developing rat embryos *(12).* These results might suggest that inadequate zinc results in impaired cellular immune functions because of a decrease in DNA synthesis and a subsequent diminished capability to amplify T- and B-cells in response to foreign invasion.

Dietary Zn might also influence the immune system indirectly by interaction with growth and infectivity of organisms that are pathogens to animals. Zn deficiency in animals is associated with increased infections with micro-organisms and causes Gram-negative sepsis in rats by increased bacterial populations present in liver, lungs, and kidneys *(21).* Chickens have hypozincemia when infected with the Newcastle disease virus *(22)* or *Escherichia coli* endotoxin *(23).* Zinc concentrations in the liver are increased by *E. coli* endotoxin infection *(24).* Temporal and quantitative changes in zinc concentrations in immune tissues might be important in the response to infection because the host uses zinc as a cofactor for enzymes involved in defense against pathogens *(24,25).* Increased liver weight by infection could be the result of interleukin (IL-1) stimulation of acute-phase reactant protein synthesis such as metallothionein and ceruloplasmin *(24,25).* Zn concentrations in serum and plasma are initially depressed when birds are infected with *Salmonella gallinarum (26), E. coli (27),* or *E. coli* endotoxin *(23).* The effectiveness of Zn inhibition of bacterial growth results from changing the active transport system and impeding the initial phase of bacterial mating *(28).* They also propose that zinc treatments such as zinc chloride enhanced survival incidence in rats infected with *Francisella tularensis* or *Streptococcus pneumoniae.* This can be explained by zinc's association with defense mechanisms such as leukocytosis, phagocytosis, and cell-mediated immunity as well as indirectly by zinc's inhibition of proliferation of these foreign invaders.

ZINC BIOLOGICAL UPTAKE

Bacterial Zinc Transport

The biological processes involved in bacterial transport and resistance have been reviewed in extensive detail in refs. *29* and *30.* The following is a brief overview of the more recent findings. Bacteria can maintain zinc homeostasis because they have specific energy-dependent uptake and efflux transport systems for zinc. However, the mechanism of bacterial zinc transport has not been completely investigated. Zinc uptake and efflux systems

for Gram-negative bacteria have been broadly grouped into three general categories, namely uptake systems involved in either low, medium, or high external Zn concentrations *(30).* At low concentrations of zinc, ATP-binding cassette (ABC) transporters as binding proteins are usually induced from periplasmic membranes in Gram-negative bacteria and allow zinc to enter the cytoplasm *(29,30).* This uptake system uses ATP hydrolysis as the energy source *(29,30).* The gene *znuA* (zinc uptake) encodes a periplasmic binding protein, *znuB* encodes an integral membrane protein, and *znu*C encodes the ATPase component of the transporter in *E. coli (31).* Specific transporters at medium concentrations of zinc have not been completely identified, but Pit (phosphate inorganic transport)-like proteins could functionally participate as cotransporters in accordance with intracellular requirements for external zinc *(30).* Conversely, Gram-positive bacteria do not possess an outer membrane, but they do possess many binding sites for divalent cations in the corresponding teichoic acids of their peptidoglycan, which can simply serve as surface sites of cation exchange *(30).*

At high concentrations, zinc disrupts the homeostatic balance of the cells, thereby exerting toxicity *(29,30).* Zn efflux systems play a critical role in protection of the cells from Zn toxicity. There are three different types of zinc efflux system in bacteria: CDF-type (cation diffusion facilitators) exporters; RND-type (resistance, nodulation, cell division) exporters, and P-type ATPases *(29,30).* RND-type exporters are found in *Ralstonia metallidurans (32).* The gene *czcA* (cobalt/zinc/cadmium) encodes a cation/proton antiporter for Cd^{2+}, Co^{2+}, and Zn^{2+}, and *czcB/C* encodes CzcB/C couple CzcA proteins to the outer membrane to allow extrusion of Zn^{2+} in *R. metallidurans.* CDF-type exporters are also found in *R. metallidurans (33)* and *Staphylococcus aureus (34).* The genes *czcD* and *zntA* encode cation diffusion facilitator in *R. metallidurans* and *S. aureus,* respectively. P-Type ATPases, named for the phosphorylated aspartate enzyme intermediate, possess a cation-transporting membrane protein found in both eukaryotes and prokaryotes *(30).* P-Type ATPases specifically transport cations (Zn^{2+}, Cd^{2+}, and Pb^{2}) into or out of a cell through the cellular membrane *(35,36).* The common characteristics include the utilization of energy derived from hydrolysis of the pyrophosphate bond of ATP for transport of cations across the cell membrane and energy state catalytic cycle involving a phosphorylated acyl intermediate *(35,36).* ATP-dependent phosphorlation at a site on the cytoplasmic side of the protein is coupled with binding and contact of one or more intracellular cations *(35,36).* This generates a high-energy intermediate and induces conformational changes causing translocation of the cation across the membrane *(37).* The CzcD protein of *Bacillus subtilis,* a CDF protein, uses an antiporter mechanism, catalyzing active efflux of Zn^{2+} in exchange for K^{+} and H^{+} *(38).*

In bacteria, the capacity for K^{+} uptake found in antiporters catalyzes the efflux of toxic monovalent or divalent cation substrates from the cytoplasm *(38).* This capacity in CzcD protein might enhance its effectiveness in conferring resistance to the toxic metals *(38).* RND proteins have two different subproteins: the MFP (membrane fusion proteins), which is perhaps local-

ized in the periplasmic space and the OMF (outer membrane factor) proteins, which facilitate passage of substrates into external medium *(39,40).* RND proteins are regarded as proton-motive force-dependent transporters because no ATP-binding site has been identified in their sequences and because the reduced accumulation of substrates in the cells was restored by the addition of inhibitors of the cytoplasmic membrane proton gradient *(29,41,42).*

Intestinal Zinc Absorption

Intestinal zinc absorption is a carrier mediated step of facilitated diffusion, which is not an energy-dependent process, but the exact uptake mechanism has not been investigated *(43,44).* There are two steps of zinc uptake as a function of the luminal zinc concentration in rats. Based on experimental observations, a rapid mucosal uptake step across the brush-border membrane was followed by a slower step that might have involved transport of zinc across the basolateral membrane *(45,46).* These authors hypothesized that the rapid uptake step in association with increasing luminal zinc concentrations might represent saturability of binding sites on the brush-border membrane. Consequently, the membranes at high concentrations might become leaky and allow zinc to enter the cell and bind nonspecifically to cell proteins and other ligands. However, the chemical form of transported zinc is not known and uptake at the membrane surface via an unidentified receptor on the brush-border surface might require binding of free ions or prior binding to specific compounds.

Intestinal zinc absorption is affected by dietary levels of zinc, the presence of other minerals in the intestinal lumen, availability of zinc chelating agents in the diet, and the synthesis of zinc carrier molecule(s) in the mucosal cells of small intestine *(47).* Zinc uptake by high-molecular-weight proteins in the intestinal mucosa is an active process requiring ATP *(44).* A low-molecular-weight zinc-binding ligand (10^5 LMW-ZBL) as a zinc transport carrier in the diet might stimulate zinc absorption *(43,48)* although the role of LMW-ZBL in stimulating zinc absorption is not well understood. An LMW-ZBL not only increases the bioavailability of zinc but also participates in carrying zinc across the mucosal cells of small intestine *(48).* Chelation by LMW-ZBL is required for zinc to absorb through small intestine. Because the free zinc ion forms an insoluble zinc hydroxide at neutral pH and the zinc transport rate is not dependent on the intraluminal zinc concentrations, zinc is transported across epithelial calls through a zinc carrier molecule *(43).* Therefore, ZBL plays a key role in regulating the intestinal zinc transport mechanism. Ethylenediaminetetraaceticacid (EDTA) has a high affinity for zinc *(50)* and it has been demonstrated to enhance zinc absorption in animals *(50–52).* EDTA chelates zinc and enhances zinc absorption in chicks *(53).* Other potential zinc transport carriers include citrate *(48),* picolinic acid *(54),* histidine *(55),* and a metallothionein *(56).*

ZINC AND POULTRY METABOLISM

Zinc in Poultry Nutrition

Zinc is a trace element that is necessary for normal growth and maintenance and includes among other functions bone development, feathering, enzyme structure and function, and appetite regulation for all avian species *(57).* Zinc at 0.012–0.018% on a total-weight basis is commonly added as a supplement to all formulated poultry diets *(57,58).* Currently, there are two inorganic feed-grade zinc sources commercially used by the poultry feed industry *(57,59):* zinc oxide (ZnO: 72% Zn) and zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$: 36% Zn). Of the supplemental zinc feed, 80–90% is ZnO, which is less bioavailable for poultry than reagent-grade or feed-grade Zn sulfate *(60–62).* However, the sulfate (acid salt) is highly water soluble, allowing reactive metal ions to promote free-radical formation, which can facilitate reactions that lead to the breakdown of vitamins and ultimately to the degradation of fats and oils, decreasing the nutrient value of the diet *(57).* Oxide is less reactive, but also less bioavailable *(57).*

Dietary zinc is relatively nontoxic to animals and humans; both exhibit considerable tolerance to high intakes of zinc *(62).* However, high levels of zinc in the diet can result in reduced growth rates in chicks *(64),* lesions of the gizzard and pancreas in laying hens *(64),* high mortality in chicks *(65),* and reduced feed intake and egg production in laying hens *(66).* Zinc toxicity is responsive to supplemental copper, and both iron and zinc interfere with copper and iron metabolism *(67,68).* Two-tenths of 1% zinc fed to chicks caused reduced tissue iron and copper concentrations *(69).* Although zinc interferes with iron metabolism in chicks, iron-deficient chicks are more susceptible to the effects of zinc toxicity than are iron-adequate chicks *(65).* This is because iron might induce the synthesis of metallothionein in the liver *(70).* Metallothionein is a nonspecific metal-buffering ligand to sequester or displace zinc from normal sites *(71).*

Zinc in Poultry Immunity

The nutritional–immunological mechanistic relationship between zinc deficiency and immune response has been extensively examined in humans *(72).* The effects of zinc supplementation on the poultry immune system and infectious disease resistance have not been throughly studied although zinc nutrition has been identified with changes in immune system responses and implicated in infectious disease resistance. In particular, zinc-methionine, (Zn-Met) and cellular poultry immunity have been studied. Zn-Met is more bioavailable than Zn-Sul (sulfate) and Zn-O when fed to chicks in corn–soybean diets *(73).*

Dietary Zn-Met supplementation (80 mg/kg for old broilers and 40 mg/kg for young broilers) in the broiler diet improves immunity in the progeny of old *(74)* and young broiler breeders *(75).* The nonspecific cellular immunity of the progeny can be enhanced as a result of Zn-Met sup-

plementation *(74,75).* Antibody responses to *Salmonella pullorum* were not different between Zn-Met and Zn-O supplementation *(74).* Zn-Met supplementation enhances in vitro macrophage phagocytosis of *Salmonella enteritidis* in young turkeys *(76).* Dietary Zn-Met supplementation in the turkey diet increases activity of macrophage phagocytosis in young turkeys *(77).* Dietary Zn-Met supplementation (40 or 80 mg/kg) in the layer diet improves survival of *E. coli* challenged in old laying hens *(78).*

ZINC AND LAYING HEN REPRODUCTION

Molting

In nature, all adult avian species undergo annual bird molting to renew their feathers. This results in body weight losses up to 40% of their mass *(79)* and a pause in oviposition because of regression of the reproductive tract *(79).* In the US commercial layer industry, older hens can be artificially induced to molt before the end of a first laying cycle, rested, and entered into a second egg laying cycle *(80).* After completion of the process of induced molting, older laying hens exhibit rejuvenation of the reproductive tracts *(81).* In the US commercial egg layer industry, feed withdrawal for a period of several days is commonly practiced to induce molt. This practice is popular because it is easy to manage and is highly effective in rapidly regressing the reproductive tract to assure a complete cessation in egg production *(81,82).*

However, alternative methods have been investigated because public awareness of molt induction by the use of feed withdrawal has increased over the years *(83–88)* and the stress associated with feed withdrawal results in increased susceptibility to *S. enteritidis* infection *(89–95).* Zinc-supplemented diets have been examined as a possible alternative for induction of molt to avoid the problems associated with feed-withdrawal induced molt. Molt induction research with zinc-supplemented diets is discussed in the next subsection.

Dietary Zinc and Molt Induction

High concentrations of zinc added to poultry layer ration have been experimentally attempted as an alternative method to induce molt. Zinc at 20,000 ppm added to the diet was effective in inducing molt and generally gave results comparable, if not significantly better, than those obtained with feed removal *(96–98).* The 20,000 ppm (2%) of zinc as zinc oxide caused a complete stop of egg production within 5 d and resulted in significant improvements of production in the periods of postmolt compared with that observed immediately premolt *(96,97).* The addition of 10,000 ppm (1%) zinc as either zinc oxide or zinc acetate to the layer ration for 14 d caused egg production to decline from 60% to 0% in 6 d *(99).* Ten thousand parts per million (1%) of zinc as zinc propionate in the supplemented

diet caused a complete induce molt and egg weights from Zn propionate-fed hens was heavier than those from feed-withdrawal treatment hens *(100).* Hens fed high concentrations of zinc ceased ovulating a day sooner that hens molted by feed withdrawal *(100,101).* There were no differences in the reproductive systems between hens molted by feed withdrawal and hens molted by high dietary zinc *(100,102).* They also indicated that the effectiveness of zinc at high concentrations might be the result of depression of feed intake. However, the moderate concentrations of zinc (≤2800 ppm) were effective for suppression of hen reproduction systems *(103).*

Mechanism of Induced Molting by Dietary Zinc

The mechanism of an induction molt by the use of dietary zinc is not completely understood, but was studied in refs. *96, 99, 101,* and *102.* In most studies, it has been reported that dietary zinc at high concentrations (10,000–20,000 ppm) induce follicular atresia and cessation of laying egg by interfering with ovulation in adult chicken because this cation (Zn^{2+}) reduces feed intake to 10–15% from the normal level. However, dietary zinc at moderate concentrations (2800 ppm) in the absence of a calcium-supplemented diet has a direct suppressive effect on the reproductive organs because calcium is required for the initiation and stimulation of gonadotropin-releasing hormone-stimulated luteinizing (LH) release *(104).*

Zinc–calcium antagonism also can occur and could explain some of the effectiveness observed when dietary zinc is supplemented as a dietary molt-induction compound. Dietary zinc at high concentrations can reduce calcium utilization and dietary calcium is the first limiting mineral for ovulation during the induced molt *(105).* Hens treated with high concentrations of dietary zinc have low plasma progesterone and the sensitivity of progesterone to LH is reduced as compared to fasted hens or hens treated with a low-calcium diet *(106).* Calcium plays an important role in gonoadotropin secretion and reproduction in avian ovarian cells *(107,108).* Zinc causes calcium to fall below a critical level essential for gonadotropin production and release *(101).* The effectiveness of zinc is not related to feed consumption and body weight difference, and zinc inhibits production of luteinizing hormone (LH)-stimulated progesterone and the formation of cyclic adenosine monophosphate (cAMP) in granulosa cells of the hen's ovary *(103).* This inhibition is not caused by a toxic effect of zinc on granulosa cells of the hen ovary. The LH-stimulated progesterone in granulosa cells is dependent on the formation of cAMP and the mobilization of extracellular and intracellular calcium. Extracellular calcium is necessary for gonadotrophin-stimulated cAMP formation *(109).*

Zinc and S. enteritidis Infection During Molt

When feed is removed as is done in feed-withdrawal molt from laying hens experimentally, the birds very rapidly (within days) become sus-

ceptible to *S. enteritidis* colonization and invasion. The *S. enteritidis* colonization is not only increased in the gastrointestinal sections of the crop and the ceca, but invasion of internal organs in chickens is increased *(90,92,93,110)* and increased horizontal transfer among birds in flocks also occurs *(111).*

Emptying of the gastrointestinal tract of the laying hen during feed-withdrawal molt apparently creates a microenvironment that not only enhances *S. enteritidis* survival but also enhances pathogenicity and subsequent expression of invasion properties *(93,95).* In addition, it appears that immune response might be compromised, leading to birds which are more physiologically susceptible *(94).* Therefore, in recent years, attempts have been made to devise dietary approaches that optimize molt induction but retain gastrointestinal tract function, indigenous gastrointestinal microflora, and bird physiology *(87,94,95).*

Given the success of zinc-amended diets for molt induction, these diets have been suggested as a means to limit *S. enteritidis* colonization and organ invasion as well. Therefore, zinc-molting diets were recently investigated whether zinc feeding might have an effect of induced molt and influence the hen's *S. enteritidis* infection. Low-calcium (800 ppm Ca), low calcium–low zinc (800 ppm Ca/110 ppm Zn) *(112)* and high zinc (10,000 ppm)-zinc-containing molting diets *(113)* decreased *S. enteritidis* colonization in laying hens when compared to hens undergoing feed withdrawal. In a study involving different zinc organic salts *(113),* zinc acetate (1% Zn) appeared be more effective for inducing molt and stimulating multiple laying cycles without increasing the risk of *S. enteritidis,* whereas zinc propionate (1% Zn) feeding was consumed in smaller quantities and was generally less effective for reducing the risk of *S. enteritidis* contamination. Lower feed consumption might have also lowered production of lactic and/or volatile fatty acid in crop and/or ceca of the molted hens by the indigenous microflora *(113).* This fact was in accordance with Ricke et al. *(112),* who reported that feeding low calcium and zinc molt diets retain sufficient fermentative activity to limit *S. enteritidis* colonization and, therefore, were generally more consistent in preventing extensive *S. enteritidis* invasion.

Potential Zinc-Associated Mechanism(s) for Limiting S. enteritidis Infection During Molt

In general, it appears that zinc dietary amendments will consistently limit *S. enteritidis* colonization and invasion in vivo, but sufficient feed intake must be retained. There are several possible mechanism(s) that might come into play that explain why dietary zinc could have a role in limiting this pathogen. Certainly, retention of feed intake and subsequent support of a fully fermentative bacterial population in the crop and the ceca is important as decreases in either the fermentation activity and/or the microbial population particularly in the crop can enhance *S. enteritidis* virulence

expression *(93,95).* For low-zinc molt-induction diets, where feed intake during the molting period is greatly diminished, fermentation profiles and quantities of fermentation products approach those seen with full-fed birds *(112).* In high-zinc molt-induction diets, where feed intakes is more restricted, fermentation activity is reduced *(113),* but when microbial diversity profiles of cecal and crop samples from these birds are compared utilizing denaturing gradient gel electrophoresis (DGGE), large shifts in populations are usually not apparent in either high- or low-zinc molted hens *(114,115).* This indicates that a high dietary intake of zinc is not eliminating potential protective microflora from the gastrointestinal tract but either directly or indirectly (via reduced feed intake) is potentially decreasing fermentation activity of the indigenous microflora.

Even if feed intake is reduced, the presence of higher concentrations of zinc in the crop and cecal lumens might be of more direct influence in limiting *S. enteritidis* as well. In a series of aerobic and anaerobic in vitro studies, Park et al. *(116–118)* determined that high concentrations of zinc compounds would decrease the growth rate of a poultry isolate of *S. typhimurium.* Several factors appeared to enhance the inhibitory action of zinc. Substituting an anaerobic atmosphere for aerobic growth conditions decreased growth in general *(116)* and the addition of reductants to the media further enhanced inhibition *(116,118).* Zinc organic salts also tended to be more inhibitory than inorganic salts, suggesting an acidic pH synergism *(116)* that was confirmed when studies were conducted where initial media pH was decreased *(117,118).* However, genetic studies are required to precisely determine the potential metabolic mechanism(s) involved in *Salmonella* response to external zinc under anaerobic atmospheric conditions. In short, the presence of dietary zinc in the laying hen's gastrointestinal tract might sufficiently limit *Salmonella* growth to decrease the potential for colonization and invasion. In addition, there is some indirect evidence that zinc might also interfere with *Salmonella* attachment mechanism(s) based on chicken skin attachment studies conducted by Nayak et al. *(119).* If interference with attachment occurred in the gastrointestinal tract as well, then this would further increase the difficulties for successful *Salmonella* establishment in susceptible laying hens.

A final consequence of the presence of additional dietary zinc in the gastrointestinal tract of laying hens might lie with the beneficial interaction of zinc with the immune system. As noted previously, feed withdrawal can severely compromise the immune system of the laying hens, making it highly susceptible to pathogen infection *(94).* It is hypothetically possible that in addition to increased nutrient intake from zinc molt-induction diets that the additional zinc stimulates the immune system and the enhanced immune response leads to a less susceptible bird to *Salmonella* invasion even if colonization in the gastrointestinal tract occurs. Kidd et al. *(76)* observed that zinc–methionine supplementation increased the reduction of intravenously introduced *S. arizona* from the spleen in young turkeys and exhibited increased in vitro phagocytosis. Immune parame-

ters under these conditions need to be examined to confirm this hypothesis. Clearly, if synergism occurs between direct limitation of *Salmonella* growth by dietary zinc in the gastrointestinal tract and a more responsive immune system, molting birds under a zinc-feeding regime should be more resistant to sustained *Salmonella* infection and horizontal transmission among susceptible birds in a molting flock. More experimental work is required to define the potential contribution of each of these factors and whether there is potential additive or synergistic effects. Such understanding might lead to more efficient delivery of dietary zinc in the gastrointestinal tract that could result in lower concentrations of dietary zinc being used to avoid potential physiological and environmental problems associated with high dosages of dietary zinc.

SUMMARY AND CONCLUSIONS

Zinc plays multiple roles in poultry metabolism. At low concentrations, it serves as an essential nutrient and functions as a metal cofactor for several enzymes. Zinc also appears to be directly involved in immune cellular functions and zinc deficiencies might also have indirect consequences on the immune system by failure to limit bacterial infections. Zinc can be taken up by biological systems, and bacterial transport and efflux systems have been identified that are energy dependent and highly regulated. Intestinal uptake is carrier-mediated facilitated diffusion and the mechanism is not well understood. Zinc uptake is also influenced by the form because it is fed some forms that are less bioavailable. High concentrations of zinc are relatively nontoxic and these concentrations have proven useful as an alternative dietary approach for molt induction to initiate a second egg-laying cycle and prevent *S. enteritidis* colonization. Given that some of this effectiveness is the result of direct antagonism of calcium metabolism indicates that lower zinc concentrations might also be effective for molt induction if calcium uptake is more completely blocked. Future studies are needed to understand the intestinal absorption mechanism involved in zinc uptake and how this might be used to enhance molt induction for much lower dietary levels of zinc than those that have been examined previously.

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